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Science Response 2015/037

ASSESSMENT OF THE OCCURRENCE, DISTRIBUTION AND POTENTIAL IMPACTS OF PISCINE REOVIRUS ON THE WEST COAST OF NORTH AMERICA

1.0 Context

Fisheries and Oceans Canada (DFO) is responsible for the regulation and management of the aquaculture industry in British Columbia. These responsibilities include licensing the transfer of farmed fish from hatcheries to marine net pens. Transfers of fish into natural waters in British Columbia falls under Section 56 of the Fishery (General) Regulation (F(G)R). Under this regulation, transfers are assessed for potential risks with respect to genetic, ecological and disease impacts into the receiving waters. The British Columbia Introductions and Transfers Committee (ITC) is responsible for reviewing applications and upon recommendation for approval, a licence can be issued.

Transferring fish from land-based freshwater hatcheries to net cages in the marine environment is an integral part of the salmon farming (aquaculture) process. Concerns have been raised regarding the presence of piscine reovirus (PRV) in farmed fish on the Pacific Coast, and the potential impacts to the health of wild salmonid populations arising from the transfer of hatchery reared fish that carry this virus to marine-based aquaculture facilities.

To support the assessment of applications to transfer fish from hatcheries to net pens, DFO Fisheries and Aquaculture Management has requested that Science Branch provide science advice regarding the potential impacts of transferring PRV infected fish to the marine environment, as part of salmon farming operations, including consideration of the development of Heart and Skeletal Muscle Inflammation (HSMI) or other diseases. Given that advice is required within four weeks of Science having been asked to respond to this request, a CSAS Science Response will be developed to address the objectives outlined below.

The assessment, and advice arising from this Canadian Science Advisory Secretariat (CSAS) Regional Peer Review (RPR), will be used to inform the assessment and licensing of applications to transfer fish raised in aquaculture hatchery facilities to the marine environment.

1.1 Objectives

1. Provide a technical review of data and studies related to the presence of PRV in wild or farmed Pacific Salmon, or farmed Atlantic Salmon, on the west coast of North America.
2. Provide a technical review of data and studies related to whether the west coast isolates of PRV cause disease in Pacific salmon or farmed Atlantic Salmon.
3. Provide a technical review of evidence related to the presence of HSMI on the west coast of North America.
4. Evaluate the adequacy of current farm-based and wild monitoring practices to detect the presence of HSMI or other diseases possibly associated with PRV.

5. Recommend key considerations for future evaluations of risk posed to wild salmon through transfer of PRV positive fish to the marine environment.
6. Discuss key uncertainties related to data, studies, or evidence that has been reviewed.

This Science Response Report results from the Science Response Process of August 19, 2015 on the Assessment of Potential Impacts of Piscine Reo-virus and HSMI on the West Coast of North America.

2.0 Background

2.1 Heart and Skeletal Muscle Inflammation (HSMI)

HSMI is a disease that was first reported from farmed Atlantic Salmon (*Salmo salar*) in Norway in 1999 (Kongtorp et al. 2004). With the exception of two reports that describe diseases similar to HSMI in farmed Atlantic Salmon in Scotland (Ferguson et al. 2005) and Chile (Gonzalez 2012), this disease has only been reported from farmed Atlantic Salmon in Norway. With respect to Chile, there is no direct association between PRV and histopathological lesions of heart and skeletal muscle inflammation. Further, the lesions found in Chilean fish do not match the classical HSMI lesions, as described for Norway (Gonzalez 2012).

HSMI is currently among the top four most common salmonid aquaculture diseases in Norway, and the number of outbreaks occurring each year increased from 54 to 142 between 2004 and 2012 (Robertsen 2011; Kristoffersen, Jensen, and Jansen 2013; Taranger et al. 2015). In fact, in 2011 and 2012, outbreaks of HSMI were more common than any other disease in Norway, accounting for upwards of 150 incidences per year (Taranger et al. 2015) and reached 180 outbreaks in 2014 (see Fish Health Reports from the Norwegian Veterinary Institute, for the years 2005-2014).

Clinical signs of HSMI usually occur 5–9 months after sea-transfer, and include abnormal swimming behaviour, anorexia, and up to 20% mortality (Biering and Garseth 2012). Less commonly, HSMI has been reported in freshwater hatcheries that use seawater in their production, as well as in smolt facilities that have no contact with the sea (Biering and Garseth 2012; Annon. 2014) (see also: Fish Health Reports from the Norwegian Veterinary Institute, for the years 2005-2014).

At necropsy, blood clots within the heart cavity are a common finding; however, other signs may include: pale or greyish coloration of the heart, "ascites, yellowish or blood-filled liver, splenomegaly, and pin-prick haemorrhages in perivisceral fat" (Biering and Garseth 2012; Kongtorp, Taksdal, and Lyngoy 2004). In Norway, upon observation of clinical signs of HSMI, the diagnosis is confirmed by the microscopic observation of specific lesions in cardiac and skeletal muscle, with occasional involvement of the liver (see: Leaflet No. 58 - ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish) (Biering and Garseth 2012; Kristoffersen, Jensen, and Jansen 2013). Histological findings are myocarditis and degeneration of the epi- and endocardium, myocardial necrosis, inflammation, as well as degeneration and necrosis of red skeletal muscle in some but not all cases (Biering and Garseth 2012; Kongtorp, Taksdal, and Lyngoy 2004; McLoughlin and Graham 2007). Multifocal necrosis of hepatocytes, with no evidence of an inflammatory response has also reported and may be a secondary consequence of the cardiac lesions (Kongtorp, Taksdal, and Lyngoy 2004). Kongtorp et al. (Kongtorp et al. 2006) notes that HSMI lesions are more common and severe in cardiac tissues than in other organs. Lesions in the heart are the first to occur, and they persist for many months after other tissues have returned to their "normal state". In a study that

documented a natural outbreak of HSMI on the Norwegian coast, in all cases involving HSMI lesions in skeletal muscle, lesions were also apparent in the heart (Kongtorp et al. 2006).

Laboratory challenge studies that have used tissue homogenates from Atlantic Salmon affected with HSMI, report the development of HSMI 6 to 10 weeks post-challenge (Table 1) (Kongtorp et al. 2004; Kongtorp, Taksdal, and Lyngoy 2004; Kongtorp and Taksdal 2009; Mikalsen et al. 2012; Finstad et al. 2014). The lesions induced in these studies are consistent with those reported from HSMI outbreaks in the field (Kongtorp et al. 2004; Kongtorp and Taksdal 2009; Finstad et al. 2014).

HSMI is one of several diseases that affect the heart and in moderate to severe cases the skeletal muscle of Atlantic Salmon (Biering and Garseth 2012; Kongtorp, Taksdal, and Lyngoy 2004). For example, in Norway, other diseases that cause myocarditis and degeneration of heart muscle include Pancreas disease (PD), Cardiomyopathy Syndrome (CMS), and a recently described disease from Rainbow Trout (Olsen et al. 2015; Kongtorp, Taksdal, and Lyngoy 2004). As in HSMI, histopathological signs of PD, and of the recently described disease from Rainbow Trout, may also include skeletal muscle inflammation and degeneration (Olsen et al. 2015; Kongtorp, Taksdal, and Lyngoy 2004; McLoughlin and Graham 2007). The lesions reported in fish with HSMI are similar to those that are reported for other diseases such as PD (caused by salmonid alphavirus), CMS (caused by piscine myocarditis virus), and a recently described disease in Rainbow Trout that is associated with the presence of genetic material from a reovirus related to PRV (Kongtorp, Taksdal, and Lyngoy 2004; Olsen et al. 2015). For this reason, HSMI cannot be definitively diagnosed by histopathology, unless the affected fish on the farm also have clinical signs consistent with HSMI. As noted above, histopathology is used to confirm the diagnosis of HSMI.

2.2 Piscine Reovirus

Piscine reovirus (PRV) was first recognized in 2010 in Norway in heart and serum obtained from Atlantic Salmon that had been challenged with tissue homogenates from Atlantic Salmon showing signs of HSMI (Palacios et al. 2010). PRV has since been detected in salmonid and non-salmonid fish in the United Kingdom, Ireland, Denmark, Chile, the United States, and Canada (Biering and Garseth 2012; Garseth et al. 2013; Kibenge et al. 2013; Garver et al. 2015; Marty et al. 2014; Siah et al. 2015; Gonzalez 2012; Annon. 2014). In the North Atlantic, hosts include farmed and wild Atlantic Salmon, Sea-trout (*Salmo trutta*), Great Silver Smelt (*Argentina silus*), Atlantic Horse Mackerel (*Trachurus trachurus*), Atlantic Herring (*Clupea harengus*) and Capelin (*Mallotus villosus*) (Wiik-Nielsen et al. 2012; Garseth et al. 2013).

Piscine reovirus (PRV) is a non-enveloped, double stranded RNA virus, which is a member of the family *Reoviridae* (Palacios et al. 2010; Kibenge et al. 2013). The phylogenetic relationship between PRV and other members of this family is not resolved, as PRV does not cluster definitively with approved members of the genera *Aquareovirus* or *Orthoreovirus* (Nibert and Duncan 2013). In the literature, PRV is sometimes called piscine orthoreovirus or Atlantic salmon reovirus (Finstad et al. 2014; Mikalsen et al. 2012; Nibert and Duncan 2013; Watanabe et al. 2006); however, for the purposes of this review we will use piscine reovirus (PRV).

Attempts to culture PRV, or other virus present in HSMI affected fish, on Atlantic Salmon kidney (ASK), Chinook Salmon embryo (CHSE-214), epithelioma papulosum cyprini (EPC), fathead minnow (FHM), Rainbow Trout gonad (RTG), Common Carp brain (CCB) and fin tissue of orange-spotted grouper, *Epinephelus coioides* (GF-1) cells have failed or proven to be largely unsuccessful (Kongtorp and Taksdal 2009; Mikalsen et al. 2012; Kongtorp et al. 2006; Garver et al. 2015). For example, although Mikalsen et al. (Mikalsen et al. 2012) reported vacuolization of GF-1 cells following exposure to a 0.45 µm filtered homogenate from heart tissue of Atlantic

Salmon with HSMI, this cell line did not support the replication of virus. At the time of their study, the sequence for PRV was not known; however, subsequently these authors identified PRV genomic material in cell culture supernatants that they studied. More recently, inoculation of GF-1 cells with PRV infected Chinook Salmon tissue failed to amplify virus as monitored through quantitative RT-PCR (Garver et al. 2015). Wessel et al. (2015) reported that mixing PRV+ erythrocytes from fish with HSMI with primary culture of PRV-free Atlantic Salmon erythrocytes supported the short term growth of and the production of infectious PRV progeny, as well as the development of inclusion bodies. Although culture in primary erythrocytes appears possible, this method was only recently published and it is not clear that the culture method can produce the high quantities of purified virus needed to support laboratory challenge studies.

2.3 Relationship between PRV and HSMI

HSMI was first described and identified as an infectious disease by Kongtorp et al. (Kongtorp et al. 2004; Kongtorp, Taksdal, and Lyngoy 2004). Although several types of viral particles were visualized in HSMI lesions by electron microscopy and a viral etiology was suspected, it was not until 2010 that PRV was identified to be associated with HSMI and a molecular diagnostic test for PRV developed (Palacios et al. 2010; Watanabe et al. 2006).

In Norway, numerous challenge studies and diagnostic testing of samples from HSMI outbreaks have provided evidence towards an association between PRV and HSMI (Table 1). All of the Norwegian studies have used injection challenge with tissue homogenates derived from HSMI diseased fish or cohabitation challenge with fish with HSMI. Although some of these studies were conducted before the identification of PRV (Kongtorp et al. 2004; Kongtorp and Taksdal 2009), analysis of archived samples from these studies identified that PRV was present (Finstad et al. 2012).

PRV is also found in a high proportion of clinically healthy, wild and farmed Atlantic Salmon collected from fresh and saltwater in Norway (Palacios et al. 2010; Lovoll et al. 2010; Garseth et al. 2013). In some instances, PRV loads in wild Atlantic Salmon spawners lacking HSMI were higher than those reported from farmed Atlantic Salmon with HSMI, suggesting that factors other than high PRV loads may be required for HSMI development in the farmed fish (Garseth et al. 2013) (see also the 2014 downloadable report available at the Norwegian Veterinary Institute, Fish Health Reports)

Moreover, PRV in Norway is not only found in fish with signs of HSMI, but also in fish showing signs of other diseases such as CMS (Lovoll et al. 2010). In Norway, to our knowledge, there is only one case where HSMI has been diagnosed in the absence of PRV (Palacios et al. 2010). Some authors have reported increasing or high PRV loads with outbreaks of HSMI or increasing severity of lesions in farmed Atlantic Salmon (Lovoll et al. 2012; Palacios et al. 2010). However, the range of PRV loads in farmed fish with HSMI often overlap with those in fish without HSMI, and PRV occurred at similar loads in cohorts of pre-smolts that remained disease free, as compared to cohorts that developed HSMI (Lovoll et al. 2012). Lovoll et al. (2012) states that "environmental factors associated with seawater locations are more important than PRV status in pre-smolts, as early exposure to virus and high viral loads do not always result in adult fish developing HSMI". Furthermore, based on a recent analysis of archived samples, PRV was present in Norwegian farmed Atlantic Salmon as early as 1988, which is 11 years before HSMI was first recognized (Dr. Espen Rimstad, Norwegian Reference Centre for Laboratory Animal Science and Alternatives, Norwegian School of Veterinary Science, Oslo, Norway, pers. comm.).

More recently, a novel calicivirus (named Atlantic Salmon calicivirus – ASCV) was cultured from heart tissue homogenates obtained from Atlantic Salmon diagnosed with HSMI (Mikalsen, et al. 2014a; Mikalsen et al. 2014b). Subsequent screening of samples from farmed salmon showed presence of ASCV in fish suffering from HSMI and CMS. Experimental challenge of Atlantic Salmon with ASCV resulted in systemic infections and histological changes indiscernible from those apparent in HSMI diseased fish. However, in these challenged fish, PRV was also observed at late stages, when the most prominent histopathological changes were seen, consequently it is unclear if what role ASCV has in the development of HSMI, and if concurrent infection with PRV predisposes fish to HSMI.

In summary, there is general agreement in the scientific literature that while PRV is typically found in association with HSMI, understanding the role of PRV in the development of HSMI has been complicated by a lack of culture techniques for this virus. HSMI is often diagnosed in association with other viruses, outbreaks of other diseases, or following stressful events on salmon farms in Norway (Biering and Garseth 2012). Attempts to culture PRV have been unsuccessful and because the presence of PRV in tissues can only be detected by molecular (e.g. RT-PCR; [Palacios et al. 2010]) or immunohistochemical methods (Finstad et al. 2012) it is difficult to test a causal relationship between PRV and HSMI. In addition, all published, controlled laboratory studies that have reported transmission of HSMI and their associations with PRV, have challenged naïve fish with tissues or live fish with a pre-existing HSMI condition. During the course of this review, we did not identify any published challenge studies in which HSMI developed in recipient animals following PRV exposure to fish or tissues did not have HSMI. Consequently, it cannot be ruled out that co-infections with another infectious agent or agents are necessary for the development of HSMI.

3.0 Analysis

The following analysis is provided for each of the objectives identified in Section 1.0.

3.1 Technical review of data and studies related to the presence of PRV in wild or farmed Pacific Salmon, or farmed Atlantic Salmon, on the west coast of North America.

PRV was first detected on the West Coast of North America in farmed Atlantic Salmon through RT-PCR tests on audit samples that were conducted in 2010 (Marty and Bidulka 2013). Since that time, molecular surveillance work through various labs and agencies in Canada and the United States (Washington State and Alaska) has expanded the known host range of PRV to include: Cutthroat Trout (*Oncorhynchus clarkii*), wild and farmed Chinook Salmon (*Oncorhynchus tshawytscha*), Sockeye Salmon (*Oncorhynchus nerka*), Steelhead Trout (*Oncorhynchus mykiss*), Coho Salmon (*Oncorhynchus kisutch*), Chum Salmon (*Oncorhynchus keta*) and farmed Atlantic Salmon (Garver et al. 2015; Marty and Bidulka 2013; Marty et al. 2014; Siah et al. submitted[1]; Meyers 2014; Miller et al. 2014).

There has been limited testing of farmed salmon for PRV in marine waters of BC (Garver et al. 2015; Marty and Bidulka 2013; Marty et al. 2014). PRV was found in approximately 80% of 146 pooled samples from 539 Atlantic Salmon, tested in the Fall of 2010 (Marty and Bidulka 2013). Further PRV was found in approximately 41% of 49 archived Atlantic Salmon histology samples from 1987 – 1994; and in 76% and 97% of 168 archived farmed Atlantic and Chinook Salmon histology samples from 2000 – 2008 (Marty et al. 2014). Since the majority of samples tested were from farmed fish submitted for diagnostic evaluation, and therefore do not represent a random sample, the actual prevalence on farms may be different than reported. There are two published reports describing the prevalence of PRV in wild fish on the West Coast of North

America (Table 2) (Marty et al. 2014; Miller et al. 2014). It is important to note that a portion of the samples that were tested by Marty et al. (Marty et al. 2014) were from fish that were submitted originally for diagnostic evaluation and that different tissues were used between these studies for diagnostic testing.

The USGS laboratory in Washington State surveyed returning adult Pacific salmonids in Washington and Alaska for PRV RNA. Samples were taken over a 2 year period and to date, 1,948 fish have been tested with plans to test an additional 300 fish to complete the study.

They have confirmed PRV RNA in Chinook and Coho Salmon from both Washington and Alaska. No Atlantic Salmon or other commercially cultured fish were tested for PRV by their laboratory (Dr. Maureen Purcell, Western Fisheries Research Center, US Geological Survey, Seattle, WA, pers. comm.).

The genome of PRV from samples obtained in British Columbia differed from PRV from the North Atlantic and Chile (Kibenge et al. 2013). Based on these genetic differences, Kibenge et al. (Kibenge et al. 2013) concluded that PRV in BC first diverged from Norwegian PRV in 2007 (+/- 1 year); however, recent testing of archived samples held by DFO's Pacific Aquatic Animal Health Section revealed that PRV has been present in salmonids on the Pacific Coast of North America for a much longer time, as tissues preserved for histopathology over the period of 1987 through to 1994 were positive for PRV, with a suspect detection in wild Steelhead Trout from 1977 (Marty et al. 2014). Detection of PRV in these archival samples was further confirmed by sequencing, as reported in Siah et al. (submitted[1]). In addition, these authors identified little genetic differentiation among PRV sequence types obtained over a 13 year period from a large geographical area (Alaska [US], British Columbia [Canada] and Washington State [US]), suggesting that the circulating virus types are relatively stable in the western North Pacific.

In summary, a North Pacific variant of PRV occurs in wild salmonids in Western Canada and the US. There is still uncertainty about the prevalence of this virus among species and life-history stages of wild Pacific salmon and among farmed salmon in Western Canada.

3.2 Technical review of data and studies related to whether the west coast isolates of PRV cause disease in Pacific Salmon or farmed Atlantic Salmon.

The presence of PRV in Western North America allows for the opportunity to study the pathogenicity of the virus in a region in which HSMI has not been reported. Garver et al. (2015) challenged Atlantic, Chinook and Sockeye Salmon by intra-peritoneal (i.p.) injection with pooled kidney and liver tissue homogenates prepared from Chinook Salmon displaying signs of Jaundice Syndrome that were also infected with PRV. A total of two fish out of 63 fish died during the 22 weeks following injection; these mortalities did not have Jaundice Syndrome, nor were they infected with PRV. Histopathological examination of fish collected at 22 weeks showed no gross or histological evidence of Jaundice Syndrome or HSMI, although all of the challenged fish tested positive for low levels PRV (median CT 32-37). These authors concluded that the West Coast strain of PRV, while transmissible, was of low pathogenicity for Chinook, Sockeye, and Atlantic Salmon.

More recently, naïve Atlantic and Sockeye Salmon were challenged with PRV under controlled laboratory conditions to systematically evaluate the transmission dynamics and whether challenge with PRV from Western North America would result in the development of HSMI or other disease conditions. Fish were challenged by i.p. injection, as well as by cohabitation with naturally infected Atlantic Salmon. The injection challenge was monitored for 24 weeks, during which fish were subjected to the physiologically demanding stage of smoltification at two weeks post challenge. This was done to capture this critical life stage and reflect the common temporal pattern of HSMI in Norway. Similarly, the fish used in the co-habitation challenge were

smolted at or near the start of the challenge and were monitored for 41 weeks (Garver et al. submitted[1].). These studies demonstrated that PRV from the West Coast of North America is infectious by injection or co-habitation challenge in Atlantic and Sockeye Salmon, with PRV persisting throughout both experiments (Garver et al. submitted[2]). Although the PRV loads obtained in this study were similar to or higher than PRV loads reported in Atlantic Salmon with HSMI (Finstad et al. 2014; Lovoll et al. 2012), no characteristic histopathological signs of HSMI occurred in: Atlantic Salmon donors from which tissue PRV+ homogenates were produced, injection challenged Atlantic Salmon collected at 6, 12, and 24 weeks post challenge, and donor and recipient Atlantic and recipient Sockeye Salmon collected at 12 and 41 weeks post challenge from the cohabitation trials. Neither were any other signs of disease identified that could be attributed to PRV infection. Comprehensive histopathological analysis was conducted by two board certified veterinary pathologists at the Animal Health Center, BC Ministry of Agriculture. Organs examined included heart, skeletal muscle, skin, liver, spleen, trunk kidney, brain, eye, intestinal ceca, and mesenteries with pancreas.

Researchers in Washington State have also conducted freshwater PRV challenge trials in Chinook Salmon and Rainbow Trout fry (Purcell, Garver, and Winton 2014; Dr. Maureen Purcell, Western Fisheries Research Center, US Geological Survey, Seattle, WA, pers. comm.). Fish were injected with filtered PRV positive tissue homogenates and sampled periodically over 3 months. Chinook Salmon were held at 12°C and Rainbow Trout were held at 15°C for challenge experiments. PRV replicated in both Chinook Salmon and Rainbow Trout, but mortality was negligible. Transient intraerythrocytic inclusion bodies formed in Chinook Salmon injected with PRV, but the hematocrit did not significantly decline. A transient hematocrit decrease in rainbow trout at 7 and 14 days post-injection was not temporally associated with increasing PRV RNA copy number, and very few intraerythrocytic inclusion bodies were observed. Histopathological examination of a limited number of samples from virus infected fish (10 Chinook Salmon and 10 Rainbow Trout) was generally non-remarkable. One Chinook Salmon injected with the PRV positive inoculum had increased pigment or melanin in the spleen at 84 days post-injection. Focal liver hypercellularity was observed in both mock and PRV injected rainbow trout at 64 and 84 days post-injection. However, there was no pathology noted in either species that was consistent with a diagnosis of HSMI.

The presence of PRV could influence how Atlantic and Pacific Salmon respond to other infectious agents, or have other impacts on their physiology and/or ecological performance. The effect of co-infection of PRV and Infectious Hematopoietic Necrosis Virus (IHNV) in Sockeye Salmon has been examined using laboratory challenges and transcript profiling by RNA Sequencing (RNA-seq) and RT-qPCR (Johnson et al. 2015). Injection challenge of Sockeye Salmon resulted in PRV replication and the development of a sustained infection, without evidence of disease; results that are the same as reported by Garver et al. (submitted[2]). When compared to controls, co-infection with PRV had no significant effect on survival of Sockeye Salmon following bath challenge with IHNV (Johnson et al. 2015). Although infection with PRV alone caused a very limited immune response at the transcript level in blood and head kidney, the presence of PRV appears to have had little to no effect on the subsequent immune response at the transcript level to IHNV in head kidney. The lack of host response to PRV in challenge results are in contrast to the well developed antiviral response reported by Dahle et al. (2015) for PRV infected erythrocytes sampled from a cohabitation trial with fish that were infected with PRV, as well as carrying HSMI.

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[1] Garver, K. A., Johnson, S. C., Polinski M. P., Bradshaw J. C., Marty, G. D., Snyman, H. N., Morrison, D. B. and J. Richard. 2015. Piscine Reovirus from North America is transmissible to Atlantic salmon and Sockeye Salmon but fails to cause Heart and Skeletal Muscle Inflammation. *PLoS One*. Submitted Sept. 2015

Miller et al. (2014) reported a weak, but significant, association between PRV infection and spawning migration losses in Chilko Lake Sockeye Salmon; however, this association was not seen in Sockeye Salmon of the Late Shuswap stock. These authors reported a "disease response" to infection with PRV in gill tissue in adult fish sampled in the marine environment, as indicated by their identification of 20 host genes, including 9 that were involved with immunity, that were significantly associated with PRV. In contrast, recent controlled laboratory studies examining the host immune response have found only small transitory changes in gene expression in blood and head kidney in juvenile Sockeye Salmon, associated with early stage PRV infections in saltwater (Johnson et al. 2015).

In summary, controlled laboratory studies in Chinook, Sockeye and Atlantic Salmon and Rainbow Trout provide good evidence that infection with the strain of PRV from the West Coast of North America (North Pacific variant) does not cause disease in these species. In these studies, PRV underwent periods of significant viral amplification and shedding which demonstrates a capacity of the North Pacific variant of the virus to complete its life cycle in either Atlantic or Pacific salmonids. The absence of associated mortality or pathology in infected groups exhibiting high viral loads also indicates that the Pacific PRV is non-pathogenic. Nevertheless, apart from an absence of disease, challenge with Pacific PRV results in similar infectivity and distribution in host tissues as that described for Norwegian PRV obtained from fish with HSMI (Finstad et al. 2014; Palacios et al. 2010; Mikalsen et al. 2012).

3.3 Technical review of evidence related to the presence of HSMI on the west coast of North America.

As described previously, the diagnosis of HSMI is based on a combination of clinical signs confirmed by histological examination of tissues. Based on the current state of knowledge, there have been no reports of HSMI in farmed or wild fish in British Columbia, Washington, or Alaska.

Marty et al. (2014) examined 404 wild and farmed salmonids for HSMI by histopathology and reported that none of these fish had lesions diagnostic for HSMI, nor were there any consistent lesions in fish that were carrying PRV. From 2010 - 2012 DFO contracted the Atlantic Veterinary College Aquatic Diagnostic Services to examine approximately 500 juvenile Sockeye Salmon from the Lower Fraser River, Strait of Georgia, and Johnstone Strait for the presence of disease by histopathology. There was no evidence of HSMI in these samples (DFO, Program for Aquaculture Regulatory Research, Nanaimo, BC, Stewart Johnson). In US waters, the presence of HSMI has not been reported in Washington or Alaska, despite these States having a long history of wild salmon surveillance (Meyers 2014).

Over the period of 2003 to 2010, the Province of BC and from 2011 to 2015 Aquaculture Management, Fisheries and Oceans Canada have examined 6,000 Atlantic Salmon by histopathology for signs of disease. These examinations have been conducted by American College of Veterinary Pathologists certified Veterinary Pathologists from a variety of external agencies/groups. During these examinations cardiomyopathy has been sporadically reported. Cardiomyopathy is a general term used to indicate diseases of the heart muscle; when the disease is of an unknown cause, it is called idiopathic cardiomyopathy, and it is not the same as the disease CMS that is associated with Piscine Myocarditis Virus (PMCV). Heart lesions described as cardiomyopathy have also been reported in harvest size farmed salmon in BC waters (Brocklebank and Raverty 2002).

Histological signs of idiopathic cardiomyopathy have been diagnosed as the probable cause of death in about 2% of BC Atlantic Salmon Audit Program samples examined between 2006 and-2011 and 2014 and 2015 (Dr. Gary Marty, BC Ministry of Agriculture, Animal Health Centre, Abbotsford, BC and Ian Keith, Fisheries and Oceans Canada, Fisheries Management,

Aquaculture Management Division, Courtenay, BC, pers. comms.). For example, HSMI-like lesions consisting of "endocarditis, multifocal, with endothelial cell hypertrophy", of moderate severity were first diagnosed as the probable cause of death 9 years ago (case 2006-1022; Cohen Commission Exhibit #1678). Pathologists examining Audit Program samples from 2012 – 2013 diagnosed similar lesions, but their analysis did not include cause of death determination.

When idiopathic cardiomyopathy is diagnosed as a cause of death in these audits, it usually affects only one or two fish out of 4 to 10 examined during the audit. Audits in which more than half of the examined fish died of idiopathic cardiomyopathy are rare: none from 2003 – 2010, one audit in 2011 and one audit in 2012. In none of the audits from 2013 to 2015 have more than half of the sampled fish died from idiopathic cardiomyopathy. Timing of audits is random, rather than targeted to disease outbreaks, so the cases with a higher proportion of individual fish with diagnostic lesions within a farm sample are cases where we confidently classify a farm as positive for idiopathic cardiomyopathy. Epidemiologic studies to evaluate the dynamics of disease based on proportion of individual fish with diagnostic lesions have not been performed nor have laboratory studies to rule out non-infectious etiology. However, with evidence that PRV prevalence has been greater than 50% in farmed Atlantic Salmon since at least 2000, the rare occurrence of idiopathic cardiomyopathy, since 2006, does not support the hypothesis that PRV is the cause.

Because skeletal muscle was not sampled as part of the Audit Program until 2013, only since 2013 have we been able to determine that few cases of idiopathic cardiomyopathy in BC match the pattern of microscopic lesions associated HSMI in Norway. For example, of the 1,013 Audit Program Atlantic Salmon sampled from 2014 and 2015, only two of the fish (0.2%) had both moderate skeletal muscle inflammation and significant cardiomyopathy.

Although some pathologists have summarized lesions present in samples from the audit program as "HSMI-like" or "consistent with HSMI", these diagnoses have not been consistent with a clinical pattern that matches HSMI. Infectious causes of cardiomyopathy in European farmed Atlantic salmon (e.g. the viruses causing CMS and PD) have not been found in BC despite regular PCR testing.

A retrospective analysis, of test results for PRV of 2009 Audit Program samples, found PRV to be common and not associated with any cause of mortality, including idiopathic cardiomyopathy (Marty and Bidulka 2013). Based on this analysis, and the consistent low prevalence of idiopathic cardiomyopathy that is present in the absence of HSMI-like clinical disease, no further tests for PRV have been conducted of Audit Program samples.

In summary, there is no combined clinical and histological evidence for the occurrence of HSMI in farmed salmonids in BC. There is a low prevalence of idiopathic cardiomyopathies of unknown cause(s) in audit samples of farmed salmonids in BC. With inclusion of muscle tissue in the audit samples, 0.2% of the fish examined since 2014 had signs of significant inflammation of both the heart and skeletal muscle. If we assume these lesions are caused by an infectious agent, this low percentage of infected fish suggests that it is not a highly infectious disease.

3.4 Evaluation of the adequacy of current farm-based and wild monitoring practices to detect the presence of HSMI or other diseases possibly associated with PRV

The DFO fish health audit program verifies industry information concerning the health and disease status of their marine salmon stocks and provides active surveillance. Operationally, there are visits by DFO fish health technicians to monitor activities, review health-related records, and collect samples from farmed fish for bacteria, virus and parasites and

determination of farm level disease events. All companies are required by licence condition to monitor the health status of their fish by standardized record and/or report, accounting for mortality on all farms. The comparison of the audit results with the company record provides confidence in the record and reports of health and disease status on all farms, a main goal of the program. These fish health data from the audit program also provide a venue to monitor the health of the farmed salmon. The sampling system employs standardized observational data collection and certified diagnostic evaluation following accepted epidemiological principles of disease surveillance. Data collected in this way are amenable to analysis and interpretation to allow detection of trends and changes that may indicate emerging disease.

The BC Fish Health Audit and Surveillance Program was initiated in November 2000, and its effectiveness was highlighted with detection of the index case of infectious hematopoietic necrosis (IHN) in 2001 (Dr. Sonja Saksida, Aquatic Animal Health Researcher, Halifax, Nova Scotia, pers. comm.). However, effective disease control incorporates several levels of surveillance. Most important for best practices is the leading role of farmers and their veterinarians to first identify and report suspicion of important diseases. For example, in 2012, veterinarians working for salmon farms submitted samples for diagnostic evaluation that led to the rapid diagnosis of IHN disease on three separate farms. Affected farms were quickly depopulated, thereby preventing the disease from spreading to other farms. Had these diseases not been identified by the farm veterinarians, the Aquaculture Management Division Audit Program would have eventually identified them. Government surveillance programs, like the Aquaculture Management Division Audit Program, are important for providing consistency and identifying trends in data, but they are not designed for the first identification of disease outbreaks.

Salmon farm veterinarians rapidly identify and report diseases of concern because they regularly submit samples for diagnostic evaluation that have the capacity to diagnose known and unknown diseases (e.g., histopathology). For example, from 2012 – 2015 salmon farm veterinarians submitted for histopathology an average of 180 cases per year to the BC Animal Health Centre, which is BC's internationally accredited veterinary diagnostic laboratory. The Animal Health Centre employs two board-certified veterinary pathologists that focus their diagnostic activities on fish. Most cases submitted by the salmon farm veterinarians include multiple organs, including the heart, from 5-10 fish. Diagnostic evaluation is requested to explain increases in mortality, to look for underlying causes of ongoing disease, or to evaluate the health status of fish being prepared for transfer. Submissions may include any life stage, from eggs to broodstock.

Diagnostic evaluations of farmed salmon conducted by the companies, the Province of BC or DFO Aquaculture Management Division are highly likely to have found evidence of HSMI in BC, assuming a similar presentation of HSMI as seen in Norway (described in Section 2.1). "Background noise" of heart disease in farmed Atlantic Salmon populations in other parts of the world is the norm, and in very few situations does it seem to lead to significant clinical disease (Hugh Ferguson, pathologist for 2013 audit samples). HSMI is one of the few clinical presentations of heart disease, and is peculiar to Norway. Interpretations of histologic lesions in diagnostic evaluations, where there is not an etiologic diagnosis, are not conclusive, and in British Columbia clinical signs are not seen in farms with idiopathic cardiomyopathy. Therefore, detection of heart disease relies upon mortality in an outbreak situation sufficient to be included in the fresh dead and moribund subsample, or a sample under conditions of lower dissolved oxygen. (Under conditions of low oxygen, there would not be diagnostic evaluation because the mortality would be classified as environmental.) All companies that farm Atlantic salmon in marine waters of BC marine employ a licensed veterinarian. As all these companies farm Atlantic salmon in Norway, where HSMI is common, it is unlikely that their veterinarians, other

fish health staff or managers would not be aware of the clinical signs of HSMI. In BC, all companies are required to make fish health records available to DFO Aquaculture Management Division for review and audit purposes, and submit reporting on a routine basis to Aquaculture Management Division as part of their licensing requirements.

As part of the Audit Program operated by Aquaculture Management Division in BC, about 25 Atlantic Salmon farms are monitored each quarter, as part of a health audit in which recently dead or moribund fish are collected for analysis, with the expectation that if disease, such as HSMI, were present it should be seen in these individuals. There are generally between 60 and 80 farms that are stocked with fish in each quarter. Each audit includes: examination of farm mortality records, and, complete diagnostic necropsy of the samples which are collected. A database record of the program began in 2003 with analysis of between 600- 800 fish each year, except for 2010, which was the transition year between provincial and federal oversight.

Published information on HSMI notes that inflammation and degeneration of the heart muscle is apparent in all presentations of the disease, whereas inflammation and degeneration of skeletal muscle is limited to moderate to severe cases, often resolving before heart damage resolves (Kongtorp, Taksdal, and Lyngoy 2004). In BC, salmon farm audit samples submitted for histopathological examination up to 2013 included heart along with other internal organs. Samples of pancreatic tissue have been included in these samples since 2006, and samples of and skeletal muscle have been included since 2013. Although the earlier audit samples did not include skeletal muscle for histology, this would not affect the ability to identify the presence of HSMI, as there are no presentations of this disease in which heart muscle is not involved. This is also true for samples of the wild fish that have been examined by histopathology (described above). Further, the selection of dead or moribund farmed salmon for histopathological examination increases the likelihood that disease, including HSMI if present, would be identified.

Samples collected as part of the audit process are not a random sample of farmed fish. This means that they are not appropriate for determination of pathogen prevalence within farm populations. However, they can be used to identify whether pathogens are associated with specific types of lesions.

In summary, assuming a similar clinical presentation of HSMI in BC farmed Atlantic salmon as seen in Norway, company veterinarians and/or the government audit programs would be expected to have identified HSMI if it were present.

3.5 Summary of key considerations for an evaluation of risk posed to wild salmon through transfer of PRV positive fish to the marine environment.

The following factors should be considered in any evaluation of risk posed to wild Pacific Salmon through PRV positive fish to the marine environment.

- On the West Coast of North America PRV has been found in all species of Pacific salmon, with the exception of Pink Salmon. PRV is widely distributed (Washington to Alaska) including regions where there are no salmon farms.
- Given its lack of known association with disease on the West Coast, PRV is a virus that has not been routinely tested for by Aquaculture Management Division.
- PRV has been identified in archived tissue samples from BC dating as far back as 1987, and possibly earlier.
- In Norway, PRV has been identified in some non-salmonid marine fish species. The numbers of non-salmonids on the West Coast of North America that have been tested for PRV is insufficient to address whether there are non-salmonid reservoirs of PRV.

- Injection challenge trials of Atlantic and Chinook Salmon using PRV-infected tissue homogenates obtained from BC farmed Chinook Salmon that were showing signs of jaundice, but not HSMI, did not cause HSMI, or other apparent disease over a 22 week period (Garver et al. 2015). These challenges were conducted in seawater.
- Injection challenge trials of naïve Atlantic Salmon using PRV-infected tissue homogenates obtained from farmed Atlantic Salmon without HSMI resulted in the establishment of PRV infections, but not the development of HSMI, or other disease over a 24 week period (Garver et al. submitted[2]). These challenges were conducted in seawater.
- Co-habitation challenge trials using PRV infected Atlantic Salmon not showing signs of HSMI resulted in sentinel Atlantic and Sockeye Salmon becoming infected with PRV. However, these fish did not develop HSMI, or other disease over a 41 week period (Garver et al. 2015). These challenges were conducted in seawater.
- Injection trials of Chinook Salmon and Rainbow Trout with PRV did not result in mortality or the development of HSMI (Meyers 2014) (Dr. Maureen Purcell, Western Fisheries Research Center, US Geological Survey, Seattle, WA, pers. comm.). These challenges were conducted in fresh water.
- PRV is infectious to Atlantic, Chinook, Sockeye Salmon and Rainbow Trout by injection or co-habitation. In the studies by Garver et al. (2015), PRV loads in challenged fish were generally comparable to PRV loads reported to be associated with the presence of and/or development of HSMI in Norway. Furthermore, in all cases, the duration of these experiments exceeded the time required for HSMI to develop as reported in laboratory challenges conducted in Norway.
- Assuming a similar presentation of HSMI in BC farmed Atlantic salmon as seen in Norway, veterinarians and/or the government Audit Programs would have identified HSMI if it were present.
- Over the past decade, about 2% of BC farmed salmon die each year of heart disease of unknown cause (idiopathic cardiomyopathy). However, the clinical features and microscopic lesions with these deaths do not match heart diseases described in Norway, including HSMI, CMS, PD, and the recently described disease of Rainbow Trout. More recently with inclusion of skeletal muscle in the audit samples, about 10% of these fish (0.2% of the total) have also been found to have significant inflammation of skeletal muscle, as well as heart lesions. The cause or causes of this heart disease are unknown, however, even if the 2% are dying of an infectious disease, the low prevalence supports the conclusion that the disease is not highly infectious to Atlantic Salmon.

3.6 Key uncertainties related to data, studies, or evidence that has been reviewed.

- Studies of fish diagnosed with HSMI in Norway have identified at least 4 virus-like particles by electron microscopy, 1 virus (PRV) by sequencing and immunohistochemistry, and 1 virus by cell culture (ASCV). Although most research identifies a primary role for PRV, whether any of these, or yet undiscovered agent/s, cause or contribute to the development of HSMI remains unresolved.
- There is agreement that PRV is present in farmed and wild salmon populations on the West Coast of North America collected from marine and freshwater, and that these infections are not a new occurrence. However, the prevalence of infection in different

species and populations within and among years is poorly understood for farmed and wild fish.

- Over the years, idiopathic cardiomyopathy has been reported in audit samples in British Columbia. More recently, with inclusion of skeletal muscle in the audit samples, some fish have also been found to have inflammation of skeletal muscle, as well heart lesions (e.g., two of the 1,013 Audit samples in 2014 and 2015). The cause or causes of these lesions is unknown and merits further study. Additional information is needed from the histopathologists who have examined these samples to understand how existing cardiomyopathies and muscular pathologies are differentially diagnosed from HSMI.
- There is limited information with respect to the source of PRV infection and the prevalence of PRV in commercial Atlantic Salmon hatcheries in BC.
- PRV has been identified in samples of wild adult Coho and Chinook Salmon collected at enhancement facilities. The prevalence of PRV within enhancement facilities is not known.
- The West Coast form of PRV has been shown to be transmissible by injection and cohabitation challenge. There are no data on the survival of PRV outside of its host, or on how large of an exposure to PRV (dose and time) is required to establish a waterborne infection in Atlantic and Pacific Salmon.
- PRV is commonly found in association with HSMI, as well as other disease conditions in Norway; however, the role that PRV plays in the development of HSMI in Norway remains unclear.

4.0 Conclusions

PRV occurs in populations of wild and farmed salmonids in British Columbia and in wild salmonids in US waters (Alaska and Washington State). However, information with respect to spatial and temporal occurrence of PRV in wild and farmed salmon populations and non-salmonid finfish is limited. This includes knowledge of prevalence of PRV in hatchery stocks in British Columbia.

Controlled laboratory studies demonstrated the transmissibility of the North Pacific variant of PRV among salmon species, with Chinook, Sockeye and Atlantic Salmon, as well as Rainbow Trout confirmed as being susceptible to infection. However, there is no evidence from laboratory studies in British Columbia and Washington State that PRV infection is associated any disease state, including HSMI. The studies in BC closely followed the experimental design of the Norwegian studies in that the challenge material contained PRV, but the BC studies did not use fish with clinical or histopathological signs of HSMI . HSMI has not been reported on BC salmon farms. However, idiopathic cardiomyopathy has been reported in about 2% of the audit samples in British Columbia. More recently with inclusion of skeletal muscle in the audit samples, about 10% of these fish (0.2% of the total) have also been found to have significant inflammation of skeletal muscle, as well as heart lesions. The cause or causes of these lesions are unknown, but the combination of clinical and microscopic features does not fit the diagnosis of HSMI as described in Norway. HSMI has not been diagnosed in Pacific Salmon anywhere in the world.

Based on information available, it can be concluded that the ubiquitous nature of Piscine Reovirus (PRV), its apparent long time presence in wild Pacific salmonid stocks, and the lack of clear association with disease in laboratory challenge trials, suggest a low likelihood that the

presence of this virus in any life stage of farmed Atlantic and Pacific Salmon would have a significant impact on wild Pacific Salmon populations.

British Columbia has a robust ongoing program of disease surveillance on salmon farms that integrates rapid detection of disease by licensed salmon farm veterinarians, accredited diagnostic evaluation by the BC Provincial veterinary diagnostic laboratory, detailed quarterly fish health audits by Aquaculture Management Division, and ongoing disease research by DFO Science. Together, these programs assess the impact of potential disease agents on wild stocks. Each component of this integrated program uses newly available information to minimize the threat to disease spread from farm to wild salmon.

Table 1. Summary of HSMI or PRV challenge trials from Norway and British Columbia, Canada. SW- seawater, FW – freshwater, DPC – days post challenge, WPC – weeks post challenge, IP – intraperitoneal injection, COHAB – cohabitation challenge, ND – not determined, HK – head kidney

PRV Source and Presence of HSMI	Experimental Conditions, Sample Time Points	Challenge Model, Dose	Recipient Species	First PRV Detection by Tissue, # infected/total fish examined	Signs of HSMI (cardiac)	Signs of HSMI (skeletal muscle)	Reference
PRV+ HSMI+ Atlantic Salmon	SW; 10-12°C; 1, 2, 4, 6, 8 and 10 WPC	IP, ND	Atlantic Salmon	ND	Earliest signs at 6 WPC	Minor lesions at 10 WPC	(Kongtorp et al. 2004)
PRV+ HSMI+ Atlantic Salmon	FW; 12°C; 1, 3, 4, 5, 6, 7 and 8 WPC	IP., ND	Atlantic Salmon	ND	Earliest signs at 3 WPC	ND	(Kongtorp and Taksdal 2009)
PRV+ HSMI+ Atlantic Salmon	SW; 12°C; 1, 2 , 3, 4, 5, 6, 7 and 8 WPC	IP., ND	Atlantic Salmon	ND	Earliest signs at 1 WPC	ND	(Kongtorp and Taksdal 2009)
CMS+ [a]	SW; 8.5°C; 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39 and 42 WPC	IP. ND	Atlantic Salmon	Heart – 3 WPC, 2/5; HK – 3 WPC, 2/5; Liver – 3 WPC, 1/5; Spleen – 3 WPC, 4/5	Earliest signs of CMS-type cardiac lesions at 6 WPC No HSMI lesions.	ND	(Lovoll et al. 2010)
PRV+ HSMI+ Atlantic Salmon [b]	SW; 12°C; 2 , 4, 6, 7, 8, 9 and 10 WPC	IM., ND	Atlantic Salmon	Heart – 4 WPC, 1/6; Blood – ND; HK - ND	Earliest signs at 6 WPC	Earliest sings at 7 WPC	(Mikalsen et al. 2012)
PRV+ Atlantic Salmon no HSMI	FW/SW @ 16 DPC; 11°C; 3 DPC, 1, 2, 4, 6, 12 and 24 WPC	IP.	Atlantic Salmon	Heart – ND; Blood – 3 DPC, 15/15; HK – 3 DPC; 15/15	No HSMI detected @ 12 and 24 WPC	No HSMI detected @ 12 and 24 WPCs	Garver et al. (submitted[2])
Lab Infected Atlantic Salmon with HSMI	SW; 10-12°C; 1, 2, 4, 6, 8 and 10 WPC	Cohab, ND	Atlantic Salmon	ND	Earliest signs at 10 WPC	Minor lesions at 10 WPC	(Kongtorp et al. 2004)
Lab Infected Atlantic Salmon with HSMI	SW; 12°C; 6, 8, and 10 WPC	Cohab , ND	Atlantic Salmon	Heart – 6 WPC, 100% infected; Blood – ND; HK – ND	10 WPC although minor changes observed earlier	Earliest signs at 6 WPC	(Mikalsen et al. 2012)

PRV Source and Presence of HSMI	Experimental Conditions, Sample Time Points	Challenge Model, Dose	Recipient Species	First PRV Detection by Tissue, # infected/total fish examined	Signs of HSMI (cardiac)	Signs of HSMI (skeletal muscle)	Reference
Lab Infected Atlantic Salmon with HSMI	SW[a]; 12°C; 4, 6, 8, 10, 12 and 14 WPC	Cohab (Trial 1)	Atlantic Salmon	Heart – 6 WPC, 4/6; Blood – 6 WPC, 4/6; HK – 6 WPC, 5/6; Spleen – 6 WPC, 5/6; SM – 8 WPC, 6/6	ND	ND	(Finstad et al. 2014)
Lab Infected Atlantic Salmon with HSMI	SW[a]; 12°C; 2, 3, 4, 5, 6, 7 and 8 WPC	Cohab (Trial 2)	Atlantic Salmon	Blood – 4 WPC, 3/6; RBC – 4 WPC, 1/6; Plasma – 4 WPC, 1/6; Spleen – 4 WPC, 1/6;	ND although a HSMI lesion shown from a sample collected at 7 WPC	ND	(Finstad et al. 2014)
Hatch PRV positive Atlantic Salmon no HSMI	FW/SW @ 16 DPE; 11°C; 1, 2, 4, 6, 12, 24 and 41 WPC	Cohab	Atlantic Salmon	Heart – ND; Blood – 2 WPC, 1/15; HK – 4 WPC; 15/15	No HSMI detected @ 12 and 41 weeks	No HSMI detected @ 12 and 41weeks	Garver et al. (submitted[2])
Hatch PRV positive Atlantic Salmon no HSMI	SW[c]; 11°C; 1, 2, 4, 6, 12, 24 and 41 WPC	Cohab	Sockeye Salmon	Heart – ND; Blood – 4 WPC, 4/10; HK – 4 WPC, 2/10	No HSMI detected @ 12 and 41 weeks	No HSMI detected @ 12 and 41 weeks	et al. (submitted[2])

a. Samples from Fritsvoid et al. (Fritsvold et al. 2009). Experimental transition of cardiomyopathy syndrome (CMS) in Atlantic Salmon, *Salmo salar*.

b. Samples examined for PRV were also infected with infectious pancreatic necrosis virus (IPNV).

Table 2. Prevalence of PRV in archived and fresh samples of wild Pacific Salmon collected over the period of 1994 to 2013. Data summarized from (Marty et al. 2014; Miller et al. 2014).

	# of Archived /Prevalence	# of Fresh Samples /Prevalence	Reference
Chinook (adult)	BC = 8 / 22.0%	BC = 66 / 9.9%	(Marty et al. 2014)
Coho (adult)	-	BC = 60 / 5.0% US =58 / 10.3%	(Marty et al. 2014)
Chum	-	BC = 101 / 0%	(Marty et al. 2014)
Pink (juvenile)	-	BC = 76 / 0%	(Marty et al. 2014)
Pink (adult)	-	BC = 120 / 0% US = 117 / 0%	(Marty et al. 2014)
Sockeye (juvenile)	BC = 30 / 0%	-	(Marty et al. 2014)
Sockeye (adult)	BC = 74 / 6.7%	BC = 180 / 1.7% BC = 439 / 19.4% US = 120 / 0%	(Marty et al. 2014; Miller et al. 2014)
Steelhead	BC = 10 / 30.0%	-	(Marty et al. 2014)

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